

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

SUCCESSOR TO THE
TOBACCO INDUSTRY RESEARCH COMMITTEE
633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:

Dr. Jacobson, Chm.
Dr. Cattell
Dr. Bing

Application For Research Grant

Date: May 27, 1966

1. Name of Investigator: John R. Rowlands

2. Title: Senior Research Scientist

3. Institution &
Address: Southwest Research Institute
8500 Culebra Road
San Antonio, Texas 78206

4. Project or Subject:
A detailed investigation of the nature of the reaction between biological materials and atmospheric contaminants using Electron Paramagnetic Resonances and optical spectroscopic techniques is proposed. Such a program could lead to the development of experimental methods for assessing the relative importance of various atmospheric contaminants as biological hazards.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

In recent years a great amount of statistical information has led to the belief that there exists a close relationship between cigarette smoking and lung cancer. In addition, studies of this sort have also indicated a significantly higher incidence of lung cancer in areas of high atmospheric pollution. It has so far proven to be very difficult to establish in an objective way the nature of the effects of air pollution and smoking because of the lack of a convenient experimental method.

The advent of Electron Spin Resonance spectroscopy has provided the most powerful experimental technique that is currently available for the detection and identification of both free radicals and compounds containing paramagnetic metals. The technique has been used with great success in this last decade in such diverse areas as radiation biology, chemical kinetics and providing important experimental parameters for improving ligand field theory. Instead of discussing in detail here the experimental and theoretical aspects of electron paramagnetic resonance we have included as appendices two reprints which indicate in some detail the potential of the method.

Referred for critique
from Industry
Technical
Committee.

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In the course of studies sponsored by NCI involving the chemical nature and properties of the constituents of tobacco smoke we have demonstrated that the reactive species found in the smoke interact quite dramatically with lung tissue to produce a complex electron paramagnetic resonance signal. The cigarettes were smoked directly into freshly excised rabbit lung. Details of our experimental procedure and results will be published shortly. The most interesting feature of the results is that the resonance signal we observe is not just that of a free radical signal; but consists of both a free radical signal and a resonance which may be attributed to at least one type of paramagnetic metal complex. No electron paramagnetic resonance signal was detected in control lungs under the same conditions used for the smoked lungs. The paramagnetic metal resonance signal does not exist in cigarette smoke itself and has obviously been produced by the reaction between cigarette smoke and lung tissue. We have not as yet identified the species giving rise to the signal but work is currently in progress in an attempt to make this identification. We have included as Figures 1, 2 and 3 the spin resonance signals of trapped cigarette smoke, a rabbit lung which has smoked six cigarettes and a rabbit lung which had smoked no cigarettes.

In a brief series of experiments we have also shown similar reactions of lung tissue with common atmospheric pollutant gases suggesting that a thorough investigation of the reaction between such contaminants and freshly excised lungs in addition to certain model biochemical systems such as metal-porphyrin complexes, has great promise in the study of the biological effect of atmospheric contaminants. It is suggested that the Council for Tobacco Research-U. S. A. will find in this experimental procedure a method which has the potential of allowing quantitative estimates of the biochemical (biological) effects of pollutants and further, of allowing a study of the combined biological effects of pollutants and tobacco smoke.

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6. Budget Plan:

See addenda for detailed breakdown for first year cost

- a. Salaries
- b. Expendable Supplies
- c. Other Expenses
- d. Permanent Equipment
- e. Overhead (15% of a, b, c)

Total

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available:

In addition to the senior staff listed on the cost estimate and the few small items of equipment requested in the budget all the remaining facilities and personnel are available and will be assigned as requested.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of support):

The proposed program is designed to be complimentary to the existent NCI program entitled "Physical & Chemical Properties of Free Radicals and Alkylating Agents in Tobacco Smoke," which deals explicitly with the effect of tobacco smoke alone. It is anticipated that in the latter phases of the proposed program efforts will be devoted to the study of the synergistic effects of tobacco smoke coupled with atmospheric pollutants. Together these two programs should provide an excellent experimental approach to the vexing problem to the connection between the breathing of contaminants of one form or another and the onset of lung cancers.

In order to indicate the scope of our action in the approach of electron spin resonance to a variety of chemical and biochemical problems we have appended a partial listing of sponsored programs both completed and in progress.

Signature

Director of Project

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Business Officer of the Institution

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COST ESTIMATE

First Year

A. Direct Labor Cost

J. R. Rowlands, Principle Investigator	\$ 5,460
Research Scientist and Tech. Assistants	<u>13,520</u>
Total Salaries and Wages	\$18,980

B. Material and Supplies

Animals and animal supplies	500
Miscellaneous chemicals and glassware	<u>1500</u>
	2,000

C. Other Expenses

Travel Expenses - Based on 2 Man Trips to New York	
Transportation	500
Subsistence - Based on 5 Man Days	100
Outside Consultant (Mr. Arthur Gross, Pico Laboratories) 10 days at \$125/day	1250
Photography	250
Telephone Tolls and Telegraph	<u>100</u>
	2,200

D. Surcharge

110% Salaries	20,878
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E. Special Equipment

Miscellaneous optical components for spectroscopic equipment	<u>2,000</u>
Total Estimated Cost	<u>\$46,058</u>

Signature

J. R. Rowlands
Director of Project

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I. D. Bix
Business Officer of the Institution

PREVIOUS AND PRESENT PROGRAMS

<u>Sponsor</u>	<u>Program</u>
1. Commercial Sponsor	"Free Radicals in Paper by Electron Spin Resonance," 4-7-58 to 1-7-59.
2. Quartermaster Corps	"Detection of Radiation Induced Free Radicals by Paramagnetic Resonance," Contract No. DA 19-129-QM-387, 5-18-55 to 10-18-57.
3. Quartermaster Corps and classification	"Detection of Radiation Induced Free Radicals by Paramagnetic Resonance," Contract No. 19-129-QM-1060, 11-14-57 to 7-13-59.
4. Rome Air Development Center	"Investigation of Applications of Magnetic Resonance Absorption Spectroscopy to the Study of the Effects of Microwaves in Biological Materials," Contract No. AF 30-(602)-1843, 5-1-58 to 5-1-59.
5. San Antonio R&D Procurement, Brooks AFB	"The Study of Radiation Induced Free Radicals in Chemical and Biological Systems," Contract No. AF 41(657)-246, 8-1-58 to 10-29-63.
6. Southwest Research Institute	"Basic Research—Free Radicals in Biological Materials," 8-28-57 to 1-31-58.
7. Thiokol Chemical Corporation	"Development of Rapid Methods and Techniques for Analysis of Solid Propellants Based on Nuclear and Electron Paramagnetic Resonance," 10-31-57 to 12-8-58.
8. Thiokol Chemical Corporation	"Electron Spin and Nuclear Spin Resonance Studies of PBAA Binder Systems," 12-5-58 to 3-20-60.
9. Commercial Sponsor	"Application of Magnetic Resonance Methods to the Study of Reaction Mechanisms Involving Free Radicals," 9-1-59 to 12-31-61.
10. Quartermaster Corps	"Study of Radiation Induced Reactions in Food Constituents by Resonance Methods," Contract No. DA 19-129-QM-1740, 12-30-60 to 12-29-62.

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<u>Sponsor</u>	<u>Program</u>
11. San Antonio R&D Procurement Office, Brooks Air Force Base, Texas	"The Study of Radiation Induced Free Radicals in Chemical and Biological Systems," Contract No. AF 41(657)-407, 8-1-51 to 11-30-63.
12. Commercial Sponsor	"Application of Magnetic Resonance Methods to the Study of Energy Conversion Processes of Photosynthesis," 9-1-59 to 12-31-62.
13. Commerical Sponsor	"The Mechanisms and Inhibition of Enzyme Action by Electron Spin Resonance," 5-14-62 to 5-13-63.
14. Southwest Research Institute	"An Electron Spin Resonance Study of Negative Ions of Porphyrins, 8-24-62 to 12-1-65.
15. Department of Health, Education & Welfare, Public Health Service	"Study of Radiation Damage Mechanisms to Biological Materials," 10-1-65 to 9-30-68.
16. Brooks Air Force Base, Texas	"Determination of Free Radical Content in Chemical Systems," Contract No. AF 41(609)-2771, 2-15-65 to 3-11-66.
17. Brooks Air Force Base, Texas	"The Effects of Ionizing Radiation on Oxidation States of Biological Systems," Contract No. AF 41(609)-2816, 7-1-65 to 7-31-66.
18. Food and Drug Administration	"Investigation of Chemical-Electrical Sensing Methods for the Determination of Organ Phosphate Insecticides," Contract No. RROC 663-105 (Neg.) 6-3-63 to 6-2-64.
19. U. S. Army Dugway Proving Ground	"Design and Development of Electron Capture and Flame Ionization Techniques for Tracing Biological Aerosols in the Field," Contract No. DA 42-007-AMC-82(R), 1-29-64 to 7-1-65.
20. Wright-Patterson AFB, Ohio	"Analysis of Amino Acids by Gas Chromatography," Contract No. AF 33(615)-1823, 6-10-64 to 6-15-65.

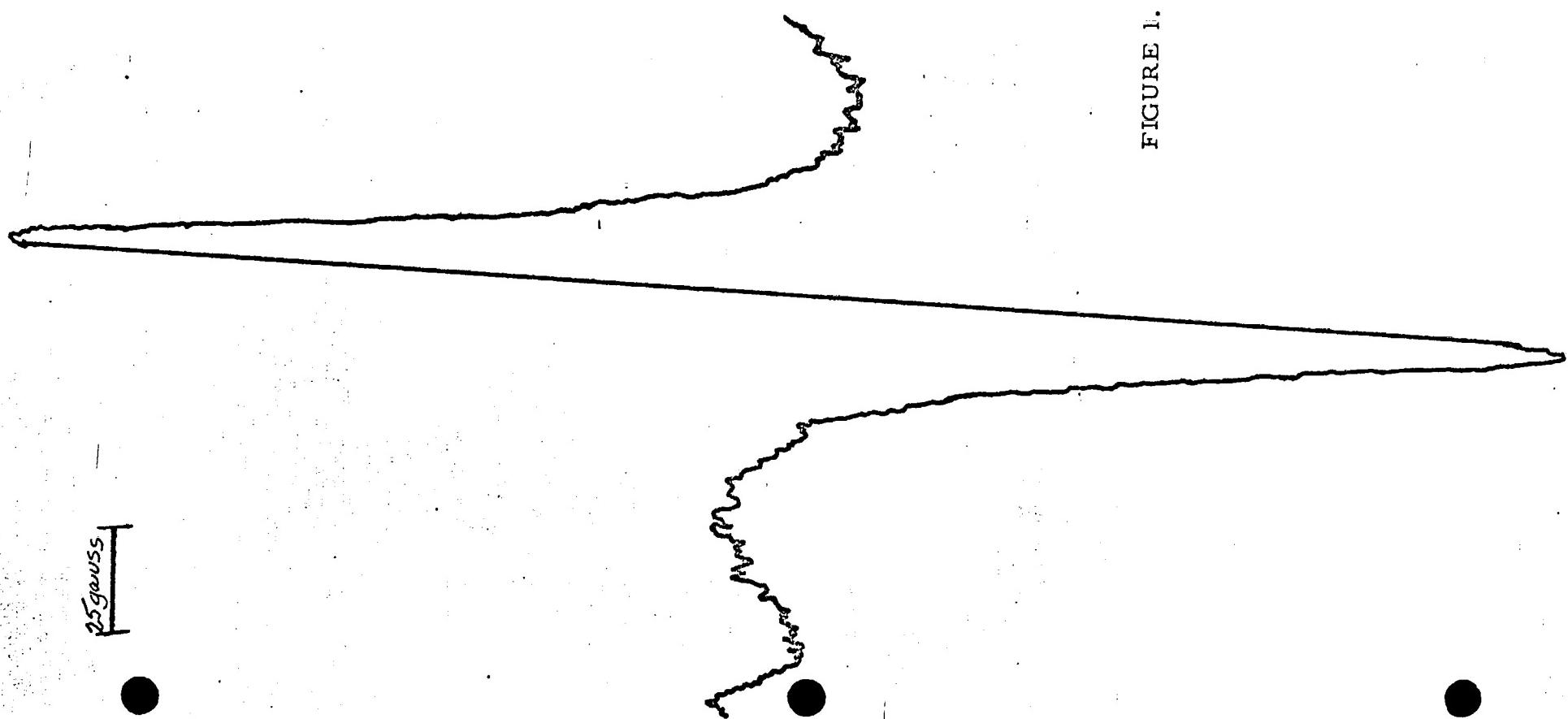
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<u>Sponsor</u>	<u>Program</u>
21. Council for Tobacco Research	"The Correlation of Acetonitrile in Body Fluids to Tobacco Usage," 4-1-64 to 3-31-65.
22. Southwest Research Institute	"Separation and Detection of Nitrogen Compounds of Biological Interest," 6-23-64 to 6-22-66.
23. U. S. Department of Agriculture	"A Study of the Basic Methods of Action of Several Fungicides Using Radio Frequency Absorption Methods," Grant No. 12-14-100-802(34), 6-28-65 to 12-28-67.
24. Public Health Service	"Radiation Damage Mechanisms in Biological Materials," Grant No. RH GM 00384-01, 1-1-65 to 9-30-68.
25. National Institutes of Health	"Physical & Chemical Properties of Free Radicals and Alkylating Agents in Tobacco Smoke," PH43-65-100, 6-23-65 to 6-22-66.

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FIGURE 1. EPR OF TRAPPED CIGARETTE SMOKE.



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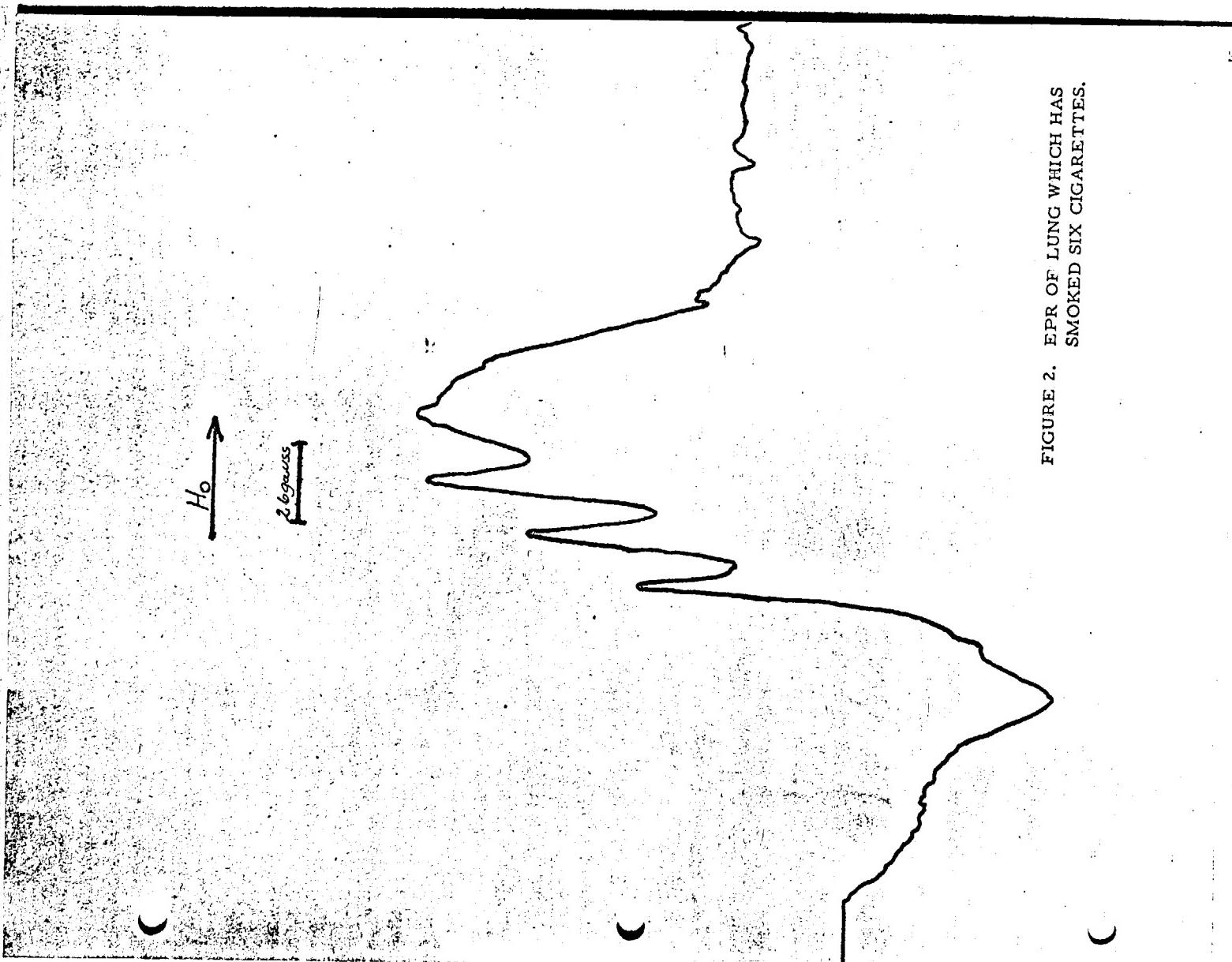


FIGURE 2. EPR OF LUNG WHICH HAS
SMOKED SIX CIGARETTES.